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LACK OF CORRELATION FOUND BETWEEN PLASMA SEX STEROIDS AND SEX STEROID-BINDING CAPACITY DURING THE ANNUAL CYCLE OF THE FEMALE COMMON CARP

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Ching-Fong Chang and Mei-Ru Chen (1991) Lack of correlation found between plasma sex steroids and sex steroid-binding capacity during the annual cycle of the female common carp. *Bull. Inst. Zool., Academia Sinica* **30**(2): 81-86. The objective of this study was to investigate the levels of sex steroids, testosterone and estradiol-17β, and sex steroid-binding capacity during the annual cycle of the female common carp, *Cyprinus carpio.* Plasma samples were obtained biweekly from 2-year-old female common carp (n=8) from November 1987 to March 1989. Sex steroid-binding capacity was measured in charcoal-treated plasma bound with tritiated steroids. Sex steroid concentrations increased during the spawning season, while no seasonal pattern of sex steroid-binding capacity was observed. No correlation was found between plasma sex steroid concentrations and sex steroid-binding capacity in the annual cycle of female common carp.

Key words: Sex steroids, Sex steroid-binding capacity, Annual cycle, Carp.

Sex steroid (testosterone and estradiol-17β)-binding proteins (SBP) have been found in the plasma of several teleosts including cod (Freeman and Idler, 1971), salmon (Freeman and Idler, 1971; Lazier *et al.*, 1985), carp (Corvol and Bardin, 1973; Chang and Chen, 1990a), brown trout (Pottinger, 1988), rainbow trout (Fostier and Breton, 1975) and goldfish (Pasmanik and Callard, 1986). The free endogenous levels of plasma steroids might be regulated by the concentrations of SBP. The relationship between the concentrations of steroids and SBP in different physiological stages of animals is of interest. For humans (Vermeulen *et al.*, 1971) and monkeys (Hodges *et al.*, 1983), SBP binding capacity decreases as the plasma testosterone (T) level increases. T levels and binding capacity of SBP show an opposite seasonal relationship in the hedgehog (Saboureau *et al.*, 1982). In alligators SBP declines during the breeding season of females, although not in males (Ho *et al.*, 1987). Pasmanik and Callard (1986) observed no seasonal differences in the binding capacity of sex steroids in goldfish. In contrast, for the brown trout the plasma testosterone-binding capacity was lowest when androgen levels were highest, and highest when androgen levels had returned to basal levels (Pottinger, 1988). No correlation between sex steroid concentrations and SBP binding capacity was found in the course of the development and annual cycle of male common

carp (Chang and Chen, 1990). In female common carp, the relationship between plasma sex steroid concentrations and sex steroid-binding capacity is unclear. Therefore, the objective of the present study was to examine the levels of plasma sex steroids and SBP during the annual cycle of the female common carp.

MATERIALS AND METHODS

A. Samples

Two-year-old adult female common carps, *Cyprinus carpio* (n=8, mean body weight=282.7±19.9 g) were reared in the aquaria (120×45×47 cm). The fishes were fed with commercial formulated food. Blood samples were collected every 2 weeks from November 1987 to March 1989. The fishes were anethetized with 2-phenoxy-ethanol and blood was drawn into heparinized tubes from the caudal vasculature. Plasma samples were stored at −20°C until the analyses of steroids and the sex steroid-binding capacity were conducted Ovarian oocytes were also aspirated biweekly with a plastic tube placed through the genital pore of the fish. The dimensions of at least 12 fresh oocytes were measured under a microscope with ocular micrometer.

B. Steroid assay

Plasma estradiol-17β (E_2) and T were measured following the solvent extraction by a validated radioimmunoassay as described by Chang and Yueh (1990). The sensitivity of the assay for E_2 and T were 10 and 12.5 pg per assay, respectively. [2,4,6,7-^{3}H] estradiol-17β (89.1 Ci/m mol) and [1, 2, 6, 7-^{3}H] testosterone (105.7 Ci/m mol) were purchased from Amersham Co., Arlington Heights, IL. Anti-E_2 serum was kindly provided by Dr. G. D. Niswender, Colorado State University (England *et al.*, 1974). Anti-T serum was generously provided by Dr. D. E. Kime, University of Sheffield, England (Kime and Manning, 1982).

C. Assessment of sex steroid-binding capacity

Plasma samples were treated with 0.5% dextran-coated charcoal solution and the mixture was then incubated at 4°C overnight (Burns and Rose, 1980; Wingfield *et al.*, 1984). The steroid-free plasma was diluted in a ratio of 1:5 with a phosphate buffer. Aliquots of 100 μl of plasma were incubated with 100 μl buffer containing 25,000 dpm of tritiated steroids (Burns and Rose, 1980; Wingfield *et al.*, 1984). Tubes were incubated overnight at 4°C. The bound steroids were separated from free the steroids by adding a dextran-coated charcoal solution (0.25% charcoal and 0.025% dextran in buffer) followed by incubating the mixtures on ice for 15 min and then centrifugating the tubes at 4°C for 5 min. Radioactivity of the bound ^{3}H-steroid contained in supernates was determined by adding 5 ml scintillation cocktail (NE 266, Nuclear Enterprises) and counting in a Beckman liquid scintillation counter (Beckman 5801). Non-specific binding control and steroid antisera control were run parallel with each assay. Non-specific binding was obtained by adding 1 μg of unlabelled steroid to displace the binding of the tritiated steroid. Specific binding was calculated by subtracting the non-specific binding from the total binding. Sex steroid-binding capacity was expressed as nM of the bound steroid. The cross reactivity of steroid binding of plasma, based on 100% binding of testosterone, with E_2, 11-ketotestosterone, 17α hydroxyprogesterone and 17α, 20β dihydroxy-4-pregnen-3-one, was 17.9%, 3.4%, 12.5% and below 0.1%, respectively.

D. Data analyses

The data were expressed as mean±

SE. Correlation coefficient between steroid concentrations and steroid-binding capacity levels were calculated. The significance of the correlation coefficient was also tested (Steel and Torrie, 1980).

RESULTS

The water temperatures in aquaria and oocyte diameters are shown in Fig. 1. Oocyte diameters remained constant throughout the experimental period. Plasma T and E_2 concentration increased from 1.0 ng/ml to 2.8 ng/ml and from 0.5 ng/ml to 1.5 ng/ml during the spawning season, respectively (Fig. 2; $p<0.05$). Plasma T- and plasma E_2-binding capacity during the annual cycle were from 0.4 nM to 1.0 nM and from 0.6 nM to 1.2 nM, respectively (Fig. 2). The correlation coefficient of plasma levels of T and T-binding capacity, and E_2 and E_2-binding capacity were 0.0340 and 0.2448, respectively. Therefore, there was no clear pattern and no correlation ($p>0.05$) between the plasma sex steroid levels and sex steroid-binding capacity in female common carp.

DISCUSSION

The diameters of the ovarian oocytes remained constant during the annual cycle. Bieniarz *et al.* (1979) showed that the diameters of large oocytes (diameter >1.1 mm) did not change. However, the diameters of small oocytes (diameter= 0.6–0.9 mm) increased during the spawning season. Yaron and Levavi-Zermonsky (1986) also observed different diameters (0.2–1.0 mm) of oocytes in the annual cycle of female common carp. The oocytes obtained with our sampling method belonged to the large oocytes category. Therefore, we observed no seasonal changes in the oocyte diameter as reported by other studies (Bieniarz *et al.*, 1979; Yaron and Levavi-Zermonsky, 1986).

Plasma levels of E_2 and T were elevated during the spawning season in in female common carp (Fig. 2). This is in agreement with steroid profiles in common carp found by Yaron and Levavi-Zermonsky (1986). Aida *et al.* (1988) showed that plasma T levels rose significantly, but plasma E_2 remained unchanged during the spawning season in

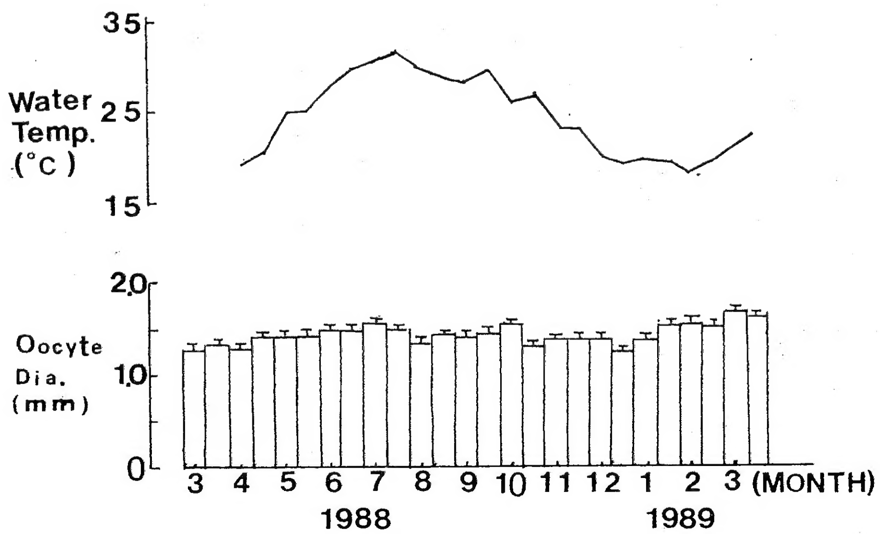

Fig. 1. Cultured water temperatures and oocyte diameter (dia.) of the female common carp ($n=8$) collected from March 1988 to March 1989.

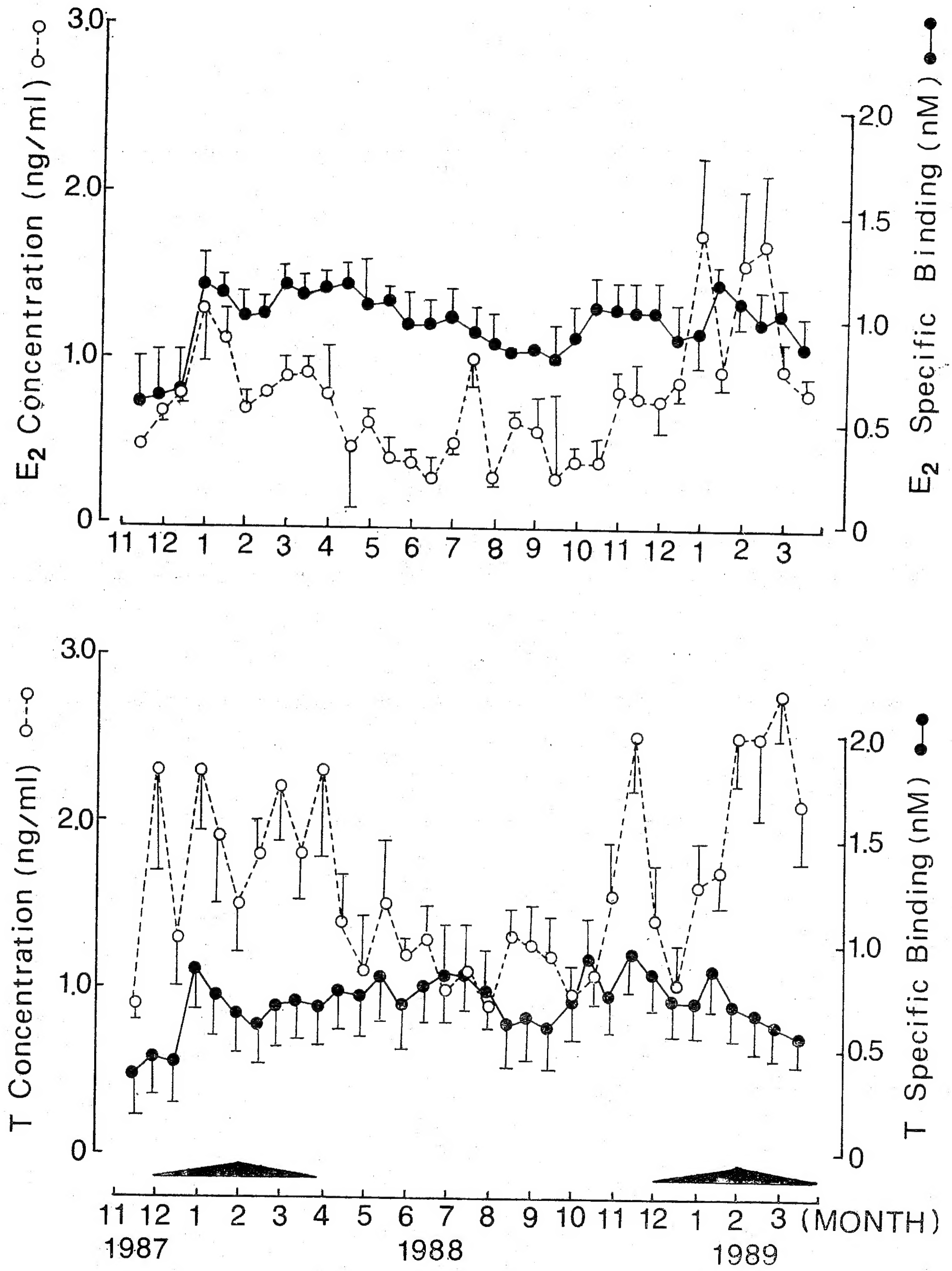

Fig. 2. Levels of plasma testosterone (T), estradiol-17β (E_2) and sex steroid (T & E_2)-binding capacity of female common carp (n=8) from November 1987 to March 1989. Dark sign (▲) indicates the spawning season.

the female common carp. Our data also showed some small peaks in the levels of plasma E_2 during the non-spawning season. High levels of plasma E_2 are necessary for vitellogenesis which is important to the ovarian recrudescence (de Vlaming *et al.*, 1980). Plasma T acts as a precursor to the synthesis of E_2 (Kagawa *et al.*, 1982).

In this study, we did not note any clear seasonal pattern of sex steroid-binding capacity in female common carp. We also did not find any correlation between sex steroid concentrations and sex steroid-binding capacity in female common carp during the annual cycle. Pasmanik and Callard (1986) observed no obvious seasonal difference in steroid-binding capacity in female goldfish. There was no correlation between sex steroid concentrations and sex steroid-binding capacity during the development of juvenile common carp and the annual cycle of male common carp (Chang and Chen, 1990). In contrast, an inverse correlation between androgen concentrations and SBP binding capacity was observed in male brown trout (Pottinger, 1988). Martin (1980) also did not observe any correlation between plasma progesterone concentrations and transcortin levels in hens. Ng and Idler (1980) suggested that gonadotropin regulates sex steroid-binding capacity in winter flounder. Martin (1980) found that thyroxine and E_2 stimulated the synthesis of transcortin in the embryonic liver of chicks. The elevation of plasma T concentrations in larval common carp occurred earlier than that of the T-binding protein (Chang and Chen, 1990b). Thus, regulation of sex steroid-binding capacity in fish is still unclear.

The physiological importance for the profiles of the sex steroid-binding capacity in female common carp is not clear. The seasonal changes of plasma steroid levels with a constant plasma steroid-binding capacity might be critical for regulating the ratio of unbound and bound plasma steroids in fish. Pasmanik and Callard (1986) demonstrated that T and E_2 interact with the same binding protein in goldfish. The biochemical characteristics of the sex steroid-binding protein in common carp are under investigation.

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雌性鯉魚年週期血液性類固醇激素之濃度與性類固醇激素結合蛋白質之結合容量並無相關

張 清 風　　陳 玫 如

本研究之目的爲探討雌性鯉魚在年週期血液中性類固醇激素之濃度 與性類固醇激素結合 蛋白質之結合容量之型態。結果顯示血液睪固酮與雌二醇之濃度在繁殖季節均有增加 ，而睪固酮與雌二醇之結合蛋白質的結合容量在年週期之過程中並無明顯的季節性變化 。 睪固酮之濃度與睪固酮結合蛋白質之結合容量並無相關性；同樣的，雌二醇之濃度與雌二醇結合蛋白質之結合容量亦無相關性。